

# Diversity of selenate-reducing bacteria in the sediment of the Olentangy River experimental wetlands: Potential reduction under anaerobic and aerobic conditions

Francoise Lucas and James T. Hollibaugh

*Department of Marine Sciences  
University of Georgia, Athens*

## Introduction

During the past decade selenium has received considerable attention from biologists and toxicologists as a result of its dual role as both an essential trace nutrient at low levels and a toxin at higher concentration (Measures and Burton, 1980). Oxidized forms are readily available for uptake by a range of marine organisms and can accumulate in the food web and generate toxicosis (Foda et al., 1983). Rivers and estuaries are susceptible to selenium contamination resulting from anthropogenic activities. These activities can cause abnormally high concentrations of trace elements to be deposited in estuarine systems and wetlands (Velinsky, 1987). Among the various sources, the effluents from agricultural drainage waters have been in focus in the mid-1980s in the western USA. Naturally high concentrations of selenium are associated with soils of marine sedimentary parent material (Losi and Frankenberger, 1997a). Agricultural irrigation of these kinds of soils has resulted in the death and deformities of waterfowl in evaporation ponds especially in San Joaquin Valley, CA (Oremland et al., 1989). A number of other anthropogenic activities, such as petroleum refining, mining and fossil fuel combustion generate Se-laden wastes (Losi and Frankenberger, 1997a).

For that reason, the development of efficient, cost-effective remediation technology for removing or immobilizing selenium from contaminated waters and sediments is of great interest. In San Francisco Bay, man-made marshes have been used for removal of selenium. Sediments can act as a sink for selenium via dissimilatory reduction in anoxic conditions. Selenate and selenite have been substantially removed from surface and interstitial waters by precipitation as elemental selenium (Oremland et al., 1989). The microbial transformation of selenium oxyanions could be a strategy for bioremediation (White et al., 1995). These biological processes include anaerobic dissimilatory reduction of selenate ( $\text{SeO}_4^{2-}$ ) and selenite ( $\text{SeO}_3^{2-}$ ) into insoluble elemental selenium ( $\text{SeO}$ ), which is nontoxic. Reduction and methylation of oxyanions under aerobic conditions, which result in production of volatile organoselenium forms, can also achieve removal (Losi and Frankenberger, 1997). A wide variety of microorganisms (pure and mixed cultures) have been studied that have the ability to reduce Se oxyanions, primarily to elemental form but also to volatile methylated forms. Until now only few

bacterial strains able to grow using selenate as the terminal acceptor of electrons have been isolated and studied (White et al., 1995). These organisms, which require anaerobic conditions for growth on selenate, are difficult to isolate and culture. Moreover, the ecological relevance and representativity of these strains are unknown.

The aim of this study was to compare the activity and the community structure of natural bacteria performing selenate-removal under anaerobic or aerobic conditions in wetland sediments. Sediment slurries were amended with selenate and the removal of selenate was followed by measuring total selenium in soluble phase. Community structure of selenate-removing populations was assessed using DGGE profiles of 16S rDNA.

## Material and methods

Surface sediment (1–2 cm deep) and water samples were collected from a wide variety of marine and freshwater sites, including a planted wetland (constructed in 1993, 1 ha) at the Olentangy River Wetland Research Park (end of October 1998). Samples were kept refrigerated until preparation of sediment slurries. The pH and salinity of the slurries were 7.9 and 1 ‰, respectively.

### *Sediment slurries*

Sediment was homogenized under  $\text{N}_2$  or  $\text{O}_2$  with wetland water (1:4 w/w) and amended with 10 mM sodium acetate and 100  $\mu\text{M}$  of sodium selenate. The homogenates were dispensed (100 ml) in 150 ml serum bottles, half were incubated under  $\text{N}_2$ , and half under  $\text{O}_2$ , at 24°C (with rotary shaking at 200 rpm). Selenate and acetate were added a second time when total dissolved selenium disappeared in dissolved phase. Subsamples were periodically withdrawn, centrifuged. Supernatants were kept frozen (–20°C) for total dissolved selenium determination by ICP. Pellets were kept frozen for DNA extraction.

### *DNA extraction*

DNA was extracted from sediment (0.3 to 0.4 g DW) according to the method described by Tsai and Olson (1991), slightly modified by adding 5% CTAB at the beginning of the extraction procedure. Crude pellets were then purified with high salt ethidium bromide treatment, phenol-chloroform extraction (Lovell and Piceno, 1994) and then concentrated with centricon 100 (Amicon) and

precipitated with ethanol.

### DNA amplification

Samples were resuspended in 15 µl of TE buffer (pH 7.2). Variable region V3 of the 16S rRNA gene was amplified by PCR with primer sequences complementary to position 341 to 358 (primer 358f, eubacterial) and positions 517 to 534 (primer 517r, universal) corresponding to *Escherichia coli* 16S rRNA numbering (Muyzer et al., 1993). A 40 bp CG clamp was added to the 358f primer: 5'-CGCCCGCCGCGCCCGCGCCCGTCCCGCCGCCCCGCCCCCGACGGGAGGCAGCAG-3'.

The 517r primer was labeled at 5'-end with fluorescein: 5'-ATTACCGCGGCTGCTGG-3'. Before PCR, the DNA was denatured for 5 min at 95°C with PCR buffer and 2.5 mM MgCl<sub>2</sub> (Promega). PCR conditions including the hot start and touchdown for primer annealing were similar to those used by Ferrari and Hollibaugh (1998, in press).

### DGGE

Electrophoresis was performed using a CBS Scientific DGGE System (Del Mar, CA). For each sample 0.5 µg of DNA (combined PCR reactions if necessary) was applied on a 6.5% polyacrylamide gel in 0.5x TAE buffer (100 mM Tris base, 12.5 mM sodium acetate, 1 mM EDTA, pH adjusted to 7.4 with acetic acid) with gradients (45-70 %), which were prepared according to Ferrari and Hollibaugh (1998). Electrophoresis was performed for 15 hours at a constant voltage of 75 V and a temperature of 60°C. After electrophoresis, the gel was read directly with the fluorescent image-scanning unit FNMIO II (Hitachi, 505 nm filter). The gel was then normalized and analyzed using the software Molecular Analyst (version 1.12, Bio-Rad). Band patterns of samples were compared by cluster analysis (Pearson correlation, UPGMA method).

## Results

### Dissolved selenium measurement

Selenium removal from dissolved phase proceeded more rapidly with N<sub>2</sub> than O<sub>2</sub> (Fig. 1). In 43 hours 50 % of dissolved selenium disappeared under anaerobic conditions, while 60 % was removed in 121 hours with aerobic respiration. A garlic-like odor characteristic of methyl derivatives of metalloids appeared rapidly in both experiments. It seems that the removal of selenium in dissolved phase occurred more rapidly after the second addition of selenate in the aerobic slurry (Fig. 1). This is also probably the case under anaerobic conditions; however, the measurements were not frequent enough.

### DGGE analysis

Bacterial community structure in samples T0 (initial condition), T4, T5 and T7 (end of selenate removal in amended slurries) were compared using the DGGE technique. Banding patterns showed strong differences between aerobic and anaerobic populations (Fig. 2). Five to 7 major bands characterized anaerobic populations, while aerobic populations exhibited 1 to 3 major bands. However, some bands were common (migration distance 201, 244 and 293). The band 201 was not present at the beginning of experiment (T0) but appeared in every selenate-removing bacterial population (Fig. 2).

According to Figure 3, the structure of the community at the initial conditions (T0) was very different from the samples showing selenate-removing activity (< 55.9% similarity). Aerobic population was more stable (89.2% of similarity between T5 and T7) than anaerobic samples (46.3% of similarity between T4 and T7). However, after the second addition of selenate, aerobic and anaerobic populations seemed to become more similar (73.6% of similarity).

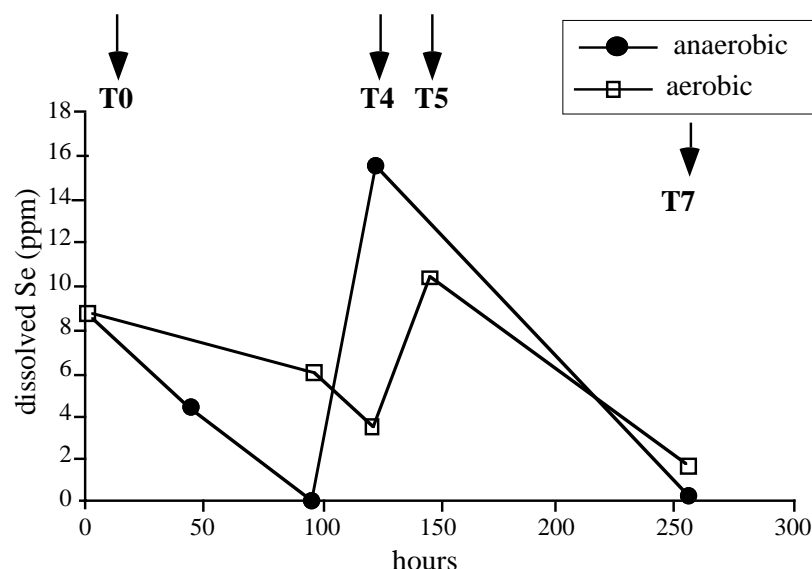


Figure 1. Evolution of total dissolved selenium in the slurries (addition of selenate at T0, T4 and T5)

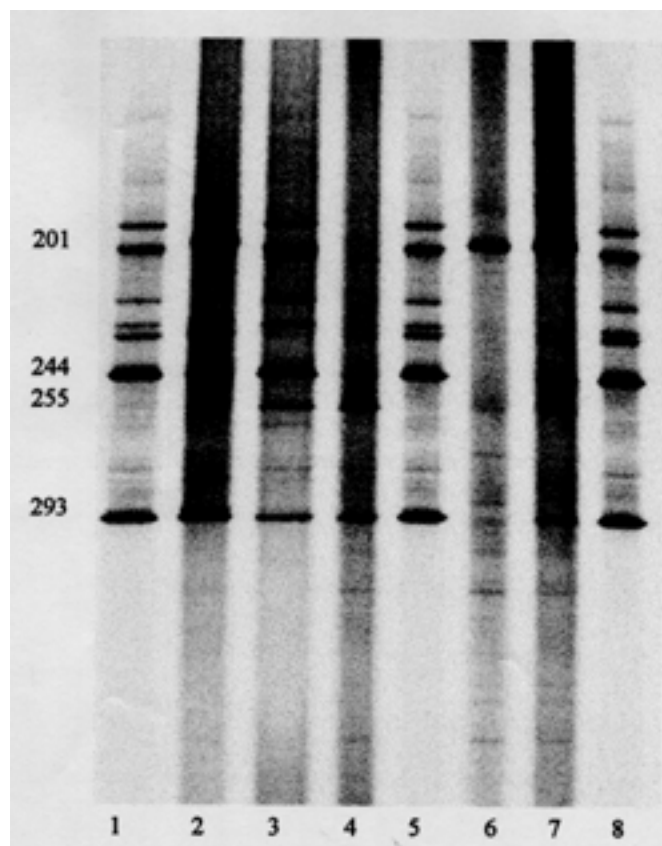


Figure 2. DGGE profiles comparing communities reducing selenate under nitrogen atmosphere or aerobic conditions. Lane 1, 5, and 8 contain a mixture of PCR template of *Clostridium perfringens* and *Bacillus thuringiensis*. Structure of the community at the original conditions: T0 (0 hour) in lane 4. Aerobic conditions: T7 (256.25 hours) in lane 2, T4 (121.08 hours) in lane 3. Aerobic conditions: T5 (145.08 hours) in lane 6, T7 (256 hours) in lane 7.

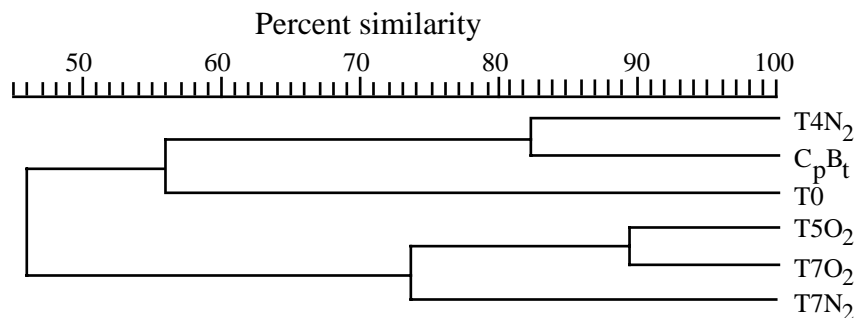


Figure 3. Cluster analysis dendrogram of the similarities (correlation, UPGMA) of the band patterns (samples). O<sub>2</sub> means aerobic conditions, N<sub>2</sub> for anaerobic conditions, CpBt for *C. perfringens* and *B. thuringiensis* mixture.

## Discussion

Selenium undergoes various biological oxidation and reduction reactions, which directly affect its toxicity and bioavailability in the environment (Losi and Frankenberger, 1997a). In the Olentangy River Wetland sediments, selenate removal from interstitial water occurred either under anaerobic or aerobic conditions. Chemical precipitation of selenate is most unlikely in these neutral-alkaline sediments, since selenate coprecipitates with aluminum or iron oxides at low pH (Lindström, 1983). Besides, a garlic-like odor characteristic of methyl derivatives of metalloids occurred

in both slurries, synonymous with selenate methylation by microorganisms (White *et al.*, 1995).

In sediment, bioreduction of selenate is performed by fungi and bacteria, resulting from various processes:

i) bioreduction to insoluble forms (SeO) or to assimilated organic forms such as seleno-amino acids;

ii) reduction and methylation, which yield volatile forms such as dimethylselenide (Losi and Frankenberger, 1997b).

It is not clear whether these pathways are independent or can occur simultaneously. Oremland *et al.* (1989) demonstrated in sediment slurries that removal of selenate under anaerobic conditions resulted from dissimilatory

reduction of selenium oxyanions with a concomitant oxidation of acetate. This activity is performed by strictly or facultative anaerobic bacteria. Certain facultative bacteria are also able to reduce  $\text{SeO}_4^{2-}$  under aerobic or microaerophilic conditions (Losi and Frankenberger, 1997b). However, these authors demonstrated that high oxygen levels inhibits selenate reduction by *Enterobacter cloacae*, but not its growth. This may explain the difference in the kinetics of selenium removal we observed in anaerobic and aerobic slurries.

The longer lag phase in aerobic slurries may also result from the shift in the community structure, since T5O2 samples displayed a bacterial community structure completely different from the one at T0. The original community in the sediments was probably more adapted to anaerobic conditions; the sediment was dark and had been collected under a layer of water. In anoxic sediment, bacteria are able to couple the oxidation of organic matter with a variety of terminal electron acceptors, including nitrate, Fe(II), Mn(IV), elemental sulfur, arsenate, selenate, and chromate (Oremland et al., 1989). Some of the bacteria present in the original community were probably able to use selenate by adapting their physiology. Indeed some bands are common between T4N2 sample and T0 (migration distance 244, 255 and 293).

The addition of selenate has selected some species adapted to the respiration of selenate and/or tolerant to selenate toxicity. For example, band 201 (relative migration distance) is only present in selenate amended samples (T4, T5 and T7) and not at the initial conditions. It may be an aerobic/anaerobic facultative bacterium, since it is present in both aerobic and anaerobic slurries. However, only the comparison of the band sequences can allow us to determine the identity of these bands migrating at the same distance.

## Conclusion

The natural bacterial community in the Olentangy River wetlands is able to reduce selenate in aerobic or anaerobic conditions. Under anaerobic conditions the sediment bacteria were more efficient, probably due to their metabolic versatility. However, under aerobic conditions, a shift occurred in the bacterial community that resulted in the selection of selenate reducing bacteria. Selenate respiration is thought to be performed by a wide range of bacterial species, although the extent of their diversity is not known. The sequencing of 16S rDNA should provide information on the diversity of selenate-reducing bacteria. The development of specific probes will allow us to screen samples for bacteria performing this activity. Selenium studies may constitute a model for the removal of other oxyanions of metalloids, such as arsenate or uranate.

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